Spectrophotometric Determination of Cephadroxil by Coupling with Diazotized p-Nitroaniline – Application to Pharmaceutical preparations

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Abstract:
A new spectrophotometric method for the assay of micro amount of cephadroxil in aqueous solution has been worked out. The method is based on the coupling of cephadroxil with diazotized p-nitroaniline in basic medium. The azo dye formed is water-soluble, stable, and shows maximum absorption at 479 nm, Beer’s law is obeyed over the range 10-200 ppm with a molar absorptivity of 2.216x10³ Lmol⁻¹ cm⁻¹, relative error of – 0.761 to +0.256% and a relative standard deviation of ±0.250 to ±2.001% depending on the concentration. The method has been applied to determine cephadroxil in pharmaceutical preparations.

Keywords: Cephadroxil, Diazotized-p-Nitroaniline, Diazotization and coupling.

Introduction:
Cephadroxil (I) is an essential pharmaceutical drug used in the elimination of bacterial infection [1].

(1)

Varies methods have been reported for the determination of cephadroxil. These include high performances liquid chromatographic technique for determination cephadroxil in pharmaceuticals and body fluids [2], atomic absorption method based on the formation of ion-pair complex between the drugs and ammonium reinekate then the formed precipitate is qualitatively determined [3]. Another method included nitration and subsequent complexation with an nucleophilic reagent [4]. A derivative spectrophotometric method used in the determination of cephadroxil in the presence of cephotaxime [5]. An oxidation of the cephadroxil with either Ce(IV) or Fe (III) in acid medium to give an intense yellow coloured product (λmax 397 nm) [6]. Another methods are based on the reaction of cephadroxil as n-electron donor with σ-acceptor iodine, and with the π-acceptors, 2,3-dichloro-5,6-dicyano-p-benzo-quinone (DDQ) and with 7,7,8,8-tetra-cyanoquinodimethane [7].

A flow injection chemiluminescence methods used in determination of cephaplsorin antibiotic (one of them cephadroxil) based on either enhance the chemiluminescence reaction of glyoxal and KMnO₄ is sulphuric acid [8], or hydrolysis of cephaplsorin with sodium hydroxide to produce sulphide ion which react with N,N-diethyl-p-phenylenediamine sulphate and Fe(III), and the blue colour produced is measured at 670nm [9]. Another method based on the luminescence increased of the tris (2,2′-bipyridine) ruthenium [11] complex with the presence of cephadroxil [10].

The present investigation describes a spectro-photometric method for the assay of cephadroxil, the method based on coupling of cephadroxil with diazotized p-nitroaniline in basic medium.

Experimental:
Apparatus:
All measurements are performed using Shimadzu UV–Visible Recording Spectrophotometric UV–160, with 1 cm matched silica cells. The pH measurements are performed on Philips PW 9420 pH meter with a combined glass electrode.

Reagents:
All chemical used are of the highest purity available.

Stock cephadroxil, 100 µg/ml. This solution is prepared by dissolved 0.01 g of cephadroxil in10 ml ethanol and the volume is completed to 100ml in a volumetric flask, the solution remains stable for two weeks at least.

Sodium hydroxide solution, IN. This solution is prepared by appropriate dilution of the concentrated volumetric (Fluka) solution with distilled water and then transferred to plastic bottle.

Diazotized p-nitroaniline reagent solution, 25 mM. A 0.3453g of a p-nitroaniline (Fluka) is dissolved in about 40 ml distilled water. Then 8 ml of 5M HCl is added and the solution is heated, the clear mixture is then transferred to a 100 ml volumetric flask and is cooled to 0 - 5°C in an ice – bath. A 0.1725g of NaNO₂ (BDH) is added and the mixture is stirred vigorously. After 5 minutes, the solution is made up to volume in 100 ml volumetric flask with cold distilled water. The solution is kept in a brown bottle in a refrigerator and is stable for 7days at least.

Cephadroxil tablets solution, 100µg/ml. Dissolve a weight of 1 tablet (average weight=0.636g of 10 tablets) from the finally powdered of cephadroxil tablets (each tablet contains 500mg cephadroxil) in 2ml of 0.1N hydrochloric acid and 10ml ethanol, shake and warm the solution. Filter the solution into a 100ml volumetric flask, wash the residue with distilled water and dilute to volume with distilled water to obtain 5000µg/ml cephadroxil A 100 µg/ml cephadroxil solution is prepared by appropriate dilution.

Cephadroxil suspension solution, 100µg/ml. The content of the container of cephadroxil suspension is dissolved in 5ml of 0.1N hydrochloric acid and 10ml of ethanol then the volume diluted to 60ml (250mg cephadroxil/5ml) with distilled water. After filtration a 2ml of solution diluted to 100ml to prepared 1000µg/ml cephadroxil. A 100µg/ml cephadroxil solution is prepared by appropriate dilution.

Varies methods have been reported for the determination of cephadroxil in aqueous solution has been worked out...
Results and Discussion:
The effect of various variables on the colour development is tested to establish the optimum conditions for determination of cephadroxil by coupling with diazotized p–nitroaniline reagent.
For the subsequent experiments, 100 μg cephadroxil is taken in 25 ml final volumes and absorbance measurements are performed at 479 nm.

Principle of the method
The method involves the coupling of the determinand with diazotized p–nitroaniline to form in basic medium an intensely–coloured dye.

Recommended Procedure and Calibration Graph
To a series of 25 ml volumetric flasks, aliquots covering the range of 10 – 200 μg cephadroxil are transferred, 4 ml of diazotized p-nitroaniline (25 mM), then 2 ml of 1 N NaOH solution are added, then the volumes are made to the mark with distilled water. The reaction mixtures are mixed and the absorbances are measured at 479 nm against the reagent blank.
Fig. 1 show that the calibration graph is linear over the range 10 – 200 μg/25ml (0.4 – 8 ppm). Higher concentration show negative deviation from Beer’s law.
The apparent molar absorptivity referred to cephadroxil, has been found to be 2.016×10^4 l mol^–1 cm^–1.

Study of the optimum reaction conditions:
The effect of various parameters on the absorption intensity of the coloured dye is investigated and the reaction conditions have been optimized.

Effect of bases:
The preliminary experiments have shown that cephadroxil give coloured dye of highest intensity with diazotized p-nitroaniline in basic medium, therefore the coupling reaction has been carried out with different (strong and weak) bases and the results show that the coloured dye need a strong basic medium, 2 ml of 1 N NaOH solution gives the highest intensity of the azo-dye, therefore this volume is recommended in the subsequent experiments (Table 1).

Table 1: Effect of base
<table>
<thead>
<tr>
<th>Base used (1N)</th>
<th>Absorbance/ml base added</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>NaOH</td>
<td>0.208</td>
</tr>
<tr>
<td>KOH</td>
<td>0.212</td>
</tr>
<tr>
<td>Na_2CO_3*</td>
<td>0.291</td>
</tr>
<tr>
<td>NaHCO_3</td>
<td>Turbid solution</td>
</tr>
</tbody>
</table>

* Turbid solution after 10 minutes.

Effect of diazotized p – nitroaniline amount:
The effect of the amount of diazotized p – nitroaniline solution (25 mM) has been studied on the dye absorbance. A 4 ml of diazotized p-nitroaniline solution in a total volume of 25 ml has been found to be sufficient due to its, highest colour intensity of the product.

Effect of time:
The coloured azo-dye developed rapidly after addition of base and immediately attained maximum intensity at room temperature. The colour is stable for at least 1 hour and the above stability period is sufficient to allow several measurements to performed sequentially.

Final absorption spectra:
Absorption spectrum of the coloured dye in basic medium, shows maximum absorption at 479 nm in contrast to reagent blank (Fig 2).

Effect of bases:
• Turbid solution after 10 minutes.

Fig. 1: Calibration graph for cephadroxil determination using diazotized p-nitroaniline reagent

Accuracy and Precision:
To check the accuracy and precision of the method, cephadroxil is determine at three different concentration. The results illustrated in Table 2 indicate that the method is satisfactory.

Table 2: Accuracy and precision of the method
<table>
<thead>
<tr>
<th>μg cephadroxil/25ml</th>
<th>Relative error, %*</th>
<th>Relative standard deviation, %*</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>-0.761</td>
<td>±1.576</td>
</tr>
<tr>
<td>100</td>
<td>±0.256</td>
<td>±2.001</td>
</tr>
<tr>
<td>160</td>
<td>-0.284</td>
<td>±0.250</td>
</tr>
</tbody>
</table>

* Average of five determinations.

Fig. 2: Absorption spectra of 100 μg cephadroxil treated according to the recommended procedure and measured against (A) blank (B) distilled water and (C) blank measured against distilled water.

The wavelength of maximum absorption at 479 nm has been selected for the subsequent experiments.
Analytical application:
The proposed method is applied to determine cephaloxin in suspension syrup and tablets cephaloxin. On applying the proposed procedure, good recovery is obtained as shown in Table 3.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Pharmaceutical preparation</th>
<th>Supplier</th>
<th>Certified value (mg)</th>
<th>μg Cephaloxin present/25ml</th>
<th>μg Cephaloxin found/25ml</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cephalaxin oral</td>
<td>Suspension syrup</td>
<td>Pharmaceutical/India</td>
<td>5.45 mg/5ml</td>
<td>86.5</td>
<td>86.3</td>
<td>99.14</td>
</tr>
<tr>
<td>Cephalaxin oral</td>
<td>Tablets</td>
<td>Ajanta Pharma Limited/India</td>
<td>5.0 mg/tablet</td>
<td>86.5</td>
<td>86.3</td>
<td>99.14</td>
</tr>
</tbody>
</table>

Table 3: Analytical applications

References: